

Detection of antigenococcal IgA in cervical secretions by indirect immunofluorescence: an evaluation as a diagnostic test

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SUMMARY Using an indirect immunofluorescent-antibody method, antigenococcal IgA was detected in the cervical secretions of 56 (72%) of 78 women with untreated gonorrhoea but in only 25 (5%) of 490 non-infected women. The low sensitivity—comparable to that of Gram-stain microscopy—the expense, and the laboriousness of the test militate against its use as a routine diagnostic procedure.

Introduction

Although gonococcal antibodies in the serum of patients with gonorrhoea may be demonstrated by various methods,¹ the detection of these antibodies has not proved useful as a diagnostic test.² A serum antibody response may not be apparent until infection has been established for several days, and thus early gonorrhoea may be missed.² Serum antibodies, particularly of the IgG class, remain detectable for weeks or months after successful treatment;³ interpretation of tests based on the demonstration of IgG antibody is therefore difficult. Wilkinson,⁴ using a fluorescent-antibody test, demonstrated significantly high titres (≥ 16) of IgM antibody in only 24 (43%) of 55 women with untreated gonorrhoea; Ison and Glynn⁵ observed that the detection of IgM antibodies by an enzyme-linked immunosorbent assay was of little diagnostic help.

Gonorrhoea is essentially an infection of mucosal surfaces, and gonococcal antibodies may be demonstrated in the urethral and cervical secretions from infected men and women respectively.^{6,7} The local IgA antibody response antedates that of the serum, and in most cases antigenococcal IgA in the secretions cannot be detected two to three weeks after successful treatment.⁷

By microscopical examination of Gram-stained smears of material from the cervix and urethra, only about 55% of women with gonorrhoea will be

identified.⁸ Culture on selective medium is the most sensitive procedure available for the diagnosis of gonorrhoea in women; however, results are not generally available until 48 hours after the collection of specimens. To control gonococcal infection within a community, a rapid and sensitive method of identifying infected, but asymptomatic, individuals is required. The purpose of this study was to determine if the detection of antibodies in cervical secretions could be used as a diagnostic test for gonorrhoea. Preliminary data are presented in this paper.

Patients and methods

STUDY POPULATION AND DIAGNOSIS

With the exception of pregnant women, all female patients who attended the Department of Sexually Transmitted Diseases, Black Street, Glasgow, and clinics held by one of us (SSRB) at the Department of Venereology, Royal Infirmary, Edinburgh, during a six-month period were studied.

The methods used in both centres for the diagnosis of gonorrhoea have previously been described.⁷

Patients were divided into three groups: (a) 78 women with untreated gonorrhoea; (b) 178 women who had previously been treated for gonorrhoea; and (c) 490 women who had no microbiological evidence of current gonococcal infection or a past history of gonorrhoea.

COLLECTION OF SPECIMENS

Under direct vision, a cottonwool-tipped applicator stick was inserted into the cervical canal to a distance

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of about 1 cm and allowed to remain in contact with the secretions for about 15 seconds. Secretions were eluted from the swab by placing this in 0.5 ml of phosphate-buffered saline, pH 7.4, containing Tween 20 (0.05% v/v). Samples were frozen at -20°C until required. After thawing and centrifugation at $2000\times g$ for 15 minutes, the supernate was used for estimating IgA and IgG concentrations and for the indirect immunofluorescent-antibody test.

ESTIMATION OF IgA AND IgG CONCENTRATIONS

Using commercially available, low-level immunodiffusion plates (Hoescht Pharmaceuticals, Hounslow, Middlesex) the concentrations of IgA and IgG in the supernate of diluted secretions from 18 patients selected at random were determined. Commercially available serum IgG (Travenol Laboratories, Norfolk) was used as reference standard; colostral IgA standard⁹ was used in the estimation of secretory IgA.

INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST

The procedure has been described in detail elsewhere.³ Strain 9 of *Neisseria gonorrhoeae*, as described by O'Reilly and co-workers,¹⁰ was used as antigen. The grading of fluorescence was as follows: 4+, brilliant fluorescence of all organisms; 3+, well defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing

organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

STATISTICAL METHOD

Statistical comparisons were made using the χ^2 test with Yates's correction.

Results

IMMUNOGLOBULIN CONCENTRATIONS

The mean IgA and IgG concentrations in the supernate of the diluted cervical secretions were 11.4×10^{-3} g/l (standard deviation, SD 6.7×10^{-3}) and 30.5×10^{-3} g/l (SD 20.6×10^{-3}) respectively.

INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST

The results obtained when cervical secretions from patients in each of the three groups were examined for IgA and IgG antibodies reactive with *N gonorrhoeae* are given in table I.

IgA

IgA antibody was detected in 56 (72%) of the 78 women with untreated gonorrhoea but in only 25 (5%) of 490 non-infected women; the mean duration of infection in the 22 women in whom antibody was not detected was 16.6 days (range 4-48 days).

Antigenococcal IgA was found in cervical secretions from 24 (22%) of 110 women who had been treated more than 28 days previously (mean interval between treatment and sampling, 422 days).

TABLE I Results of immunofluorescent-antibody test on cervical secretions from women with untreated and previously treated gonorrhoea and from non-infected patients

Antibody	No of patients		With previously treated gonorrhoea (days between treatment and sampling)				Non-infected	Total
	With untreated gonorrhoea		≤ 7	8-14	15-28	≥ 29		
IgA only	8		3	5	1	5	9	31
IgG only	7		0	12	6	16	73	114
IgA and IgG	48		10	6	4	19	16	103
None	15		1	9	11	70	392	498
Total	78		14	32	22	110	490	746

TABLE II Results of immunofluorescent-antibody test to detect antigenococcal IgA in cervical secretions from 78 women with untreated gonorrhoea compared with those of Gram-stain microscopy and culture

Fluorescent-antibody test	Positive results to direct microscopy and culture			Total
	Gram-stain only	Culture only	Gram-stain and culture	
Antigenococcal IgA detected	1	16	39	56
Antigenococcal IgA not detected	0	4	18	22
Total	1	20	57	78

IgG

IgG antibody reactive with *N gonorrhoeae* was demonstrated in the cervical secretions of 55 (71%) of 78 women with untreated gonorrhoea and in 89 (18%) of 490 non-infected patients. This antibody was found in the secretions of 19 (17%) of the 110 women who had been treated for gonorrhoea at least 29 days previously.

COMPARISON WITH GRAM-STAIN MICROSCOPY

In relation to the results of culture, the sensitivity (72%) of the fluorescent-antibody test with antiserum against IgA was almost identical to that of Gram-stain microscopy (74%) (table II).

ORAL CONTRACEPTIVES AND THE FLUORESCENT-ANTIBODY TEST

Data on the method of contraception, if any, were available for 60 women with gonorrhoea. Table III shows the results of the fluorescent-antibody test with IgA antiserum in relation to the contraceptive method used. Antigenococcal IgA was detected in 22 (73%) of 30 women who used an oral contraceptive preparation but in only 12 (40%) of 30 patients who used some other form of contraception or none. This difference in antibody detection is statistically significant ($\chi^2 = 5.50$; $P < 0.02$).

TABLE III *Influence of methods of contraception on the detection of antigenococcal IgA in cervical secretions from women with gonorrhoea*

Type of contraception	Results of fluorescent-antibody test		Total
	IgA present	IgA absent	
Oral	22	8	30
IUD or none	12	18	30
Total	34	26	60

IUD = intrauterine device

Discussion

In a previous study,⁷ IgA reactive with *N gonorrhoeae* was demonstrated in the cervical secretions (obtained by aspiration) of 71 (95%) of 75 women with untreated gonorrhoea. The rate of detection of antibody (72%) was significantly lower in the present series and probably reflects the different sampling method; the mean IgA concentration in the secretions eluted from the swabs was 11.4×10^{-3} g/l compared with 24.0×10^{-3} g/l in secretions obtained by aspiration.⁷ The collection of secretions by the latter method is however cumbersome and not suitable for routine use in a busy clinic.

The sensitivities of the immunofluorescent-antibody test with IgA antiserum and of Gram-stain microscopy were almost identical (72 and 74% respectively) but the former test was expensive and time-consuming, and results were not available for at least one hour after collection of specimens. Similar difficulties were encountered with direct fluorescent-antibody procedures.¹¹

Although antigenococcal IgA was detected in secretions from 25 (5%) of 490 women who had no microbiological evidence of gonococcal infection, it cannot be assumed that these patients had not recently been infected. In previous studies,^{7,12} IgA antibody was not detectable in the cervical secretions of women who had been treated more than 14 days previously. From the present study however, it is clear that this antibody can be detected in the secretions a month or even longer after satisfactory treatment. It is possible that some of these 25 women had been treated inadvertently before attending the clinic. As previously shown,⁷ a local antibody response may be detected in known contacts in whom repeated microbiological examination fails to show active gonococcal infection.

It was of interest that antigenococcal IgA was detected significantly more frequently in cervical fluid from women with gonorrhoea who used an oral contraceptive preparation. Chipperfield and Evans¹³ showed that the mean concentration of IgA in cervical mucus was significantly higher in women using a combined pill than in women with natural cycles.

The low sensitivity and specificity of the fluorescent-antibody test using IgG antiserum is clearly shown. IgG antibody is frequently detected in cervical secretions from patients with nongonococcal cervicitis and is probably the result of transudation of natural serum antibody through the inflamed mucus membrane.

As IgM antibody is detectable in the secretions of only about 40% of women with gonorrhoea,⁷ the fluorescent-antibody test with IgM antiserum was not performed.

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